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Interactive effects of salinity and drought stresses on soil respiration and microbial activities

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Increasing frequency of droughts and problems related to salinization in irrigated areas may directly affect soil biological functions and soil respiration. An incubation study was designed to elucidate the effects of salinity and water intercross stresses on soil respiration and microbial activities. Three different moisture levels: optimum moisture conditions (80% water holding capacity (WHC)), moderate drought (50% WHC) and severe drought stress (30% WHC) were maintained in normal (ECe=1.48 dS m^{-1}) and saline (ECe=8 dS m^{-1}) soils. Our results showed that drought stress resulted in significant increase ($p < 0.05$) in microbial biomass carbon (MBC) (601±79 mg C kg^{-1} soil) and cumulative C-CO_2 emissions (1153±96 mg C kg^{-1} soil). While under drought stress, extracellular enzyme activities showed no significant difference except leucine aminopeptidase with significant increase in its activity. Similarly, under saline conditions, MBC and soil respiration were significantly higher compared with normal soil. Extracellular enzyme activities were also significantly increased under salinity stress. In contrast, when combined stresses of drought and salinity were applied, cumulative C-CO_2 emissions were significantly decreased (968±91 mg C kg^{-1} soil) as evident by decreased β-glucosidase activity, involved in C cycling. In contrast, chitinase activity increased under combined abiotic stresses, confirming the availability of chitin from microbial dead biomass degradation. It was concluded that microbes adopted drought and salinity stresses when applied individually but combined salinity with drought stress limited microbial biomass and microbial activities in soil.

Keywords: Drought, salinity, soil respiration, microbial biomass carbon, extracellular enzyme activities.

INTRODUCTION

Global warming is among the most burning issues in the world as it is deteriorating life on earth and its ecosystem functioning. It is predicted that the mean annual global temperature at the end of this century will increase up to 2-7 ºC (Field *et al.*, 2014). This problem is mainly due to anthropogenic activities which cause increase in the atmospheric concentrations of greenhouse gases (GHGs), resulting in elevated temperature and uncertain rainfalls (Hoesly *et al.*, 2018). Due to global warming, global hydrological cycles are intensified with alternate drying and rewetting cycles, possibly transforming ecosystems (Schimel, 2018). Desertification in arid and semi-arid region, which are becoming drier due to changing rainfall pattern and water shortage, has increased. Due to these climatic changes, especially in arid and semi-arid zones, salinity is disturbing about 23% of cultivable land worldwide (Hussain *et al.*, 2019) i.e., approximately 831 million hectares are salt-affected worldwide. Hence, it is very important to understand mechanistic effects of climate change on soil salinity formation and in reverse how salinity may alter the effects of climate change (e.g., drought stress) on soil functioning?

Drought is a complex and multivariate occurrence influenced by varied physical and biological methods. Drought stress is the most common limiting factor in agricultural production, especially in arid and semi-arid regions, and there are predictions that drought stress will increase in the coming years (Pritchard, 2019). Such complication prevents simplistic explanations of cause and effect, making investigations of climate change and drought a challenging task. As the drought periods are likely to increase, then these could alter production and decomposition of soil organic matter (SOM) (Lopez-Sangil *et al.*, 2018). It has become interesting concern to study SOM acceleration or retardation due to environmental variables. Soil enzymes play vital role for nutrient mineralization and supply these nutrients to plants from soil ecosystem for their growth and production. Important soil enzymes like β-glucosidase, chitinase, phosphatase and leucine aminopeptidase responsible for C, P

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and N cycling have great relation with soil moisture. These soil enzymes are the secretions of fungi and bacteria. If the activities of fungi and bacteria are disturbed under soil drier condition, it will ultimately disturb the soil enzyme activities and nutrient cycling. Low moisture conditions due to discontinuous, scarce and erratic rainfalls are mainly responsible to control such processes in semi-arid ecosystems. A relationship is found between soil moisture and mineralization for CO_2 evolution from soil (Wang *et al.*, 2016). It is needed to know what relationship moisture and salinity have with SOM mineralization?

The increasing rate of dry periods in many regions of the world and the problems associated with salinity in irrigated areas often result in the successive occurrence of drought and salinity on farm land. Saline soils are increasing among all degraded soils and can sequester C in upper soil layer (Kashif *et al.*, 2020). Soil salinity not only affect the soil productivity but also the soil microbial processes. The effects of soil salinity on soil extracellular enzyme activities have been studied less concentrated. Several studies have been conducted to study soil enzyme activities under natural and controlled salinity stress. The most of the studies showed depressing effects of salinity on soil extracellular enzyme activities (Pan *et al.*, 2013; Shareef, 2020). The energy required by soil microorganisms to withdraw water from the soil or retain it in cells increases with decreasing osmotic potential in saline soils.

Soil salinity causes various physical changes in soil like flocculation or dispersion of soil particles and affects the solubility of SOM. High salinity limited the soil microbial activity and as a result SOM decomposition was slower (Qu *et al.*, 2019). Soil drying causes evaporation and increases the soil salinization which influences the solubility and mobility of DOC. Thus, CO_2 emissions are potentially affected under soil drying and salinity stress. It is yet not clear how salinity stress can alter microbial communities' composition and their activities. Such variations bear consequences for soil organic matter decomposition. An understanding of the effects of salinity and drought stress on soil carbon stocks and fluxes are critical in environmental management, as the areal extents of salinity and drought are predicted to increase. Keeping in view the above-mentioned facts and knowledge gaps, this study was designed for estimation of individual as well as combined impacts of salinity and drought stresses on soil respiration and microbial activities.

MATERIALS AND METHODS

***Soil sampling and preparation*:** The soil samples were collected from the top layer (0-20 cm) of agricultural fields of Faisalabad, Pakistan. After sampling, the soil was air dried and visible crop debris and small stones were removed from the soil before soil sieving with 2 mm sieve. The soil had silt loam texture (silt: 62%, clay: 22%, sand: 16%), WHC 20.5%, ECe 1.48 dS m^{-1}, Sodium adsorption ratio (SAR) 7.45 (mmol $L^{-1})^{1/2}$, pH 7.8 and SOM 0.87%. Soil salinity (ECe 8 dS m^{-1}, SAR 14 (mmol $L^{-1})^{1/2}$) was developed by following quadratic equation method. To develop desired salinity level in soil samples, the calculated quantities of the following four salts, calcium chloride ($CaCl_2$, 281 mg kg^{-1} of soil), sodium sulfate (Na_2SO_4, 704 mg kg^{-1} of soil), magnesium sulfate ($MgSO_4$, 101 mg kg^{-1} of soil) and sodium chloride (NaCl, 391.4 mg kg^{-1} of soil) were added in soil (Haider and Ghafoor, 1992).

***Soil incubation*:** An incubation experiment was conducted in controlled conditions at 20°C in the incubator. For incubation, 50 g of both normal and saline soils were taken into 200 mL incubation glass jars where three moisture levels: 80% WHC (optimum moisture), 50% WHC (moderate drought stress, and 30% WHC (severe drought stress) were maintained for 60 days. The experiment was setup under complete randomized design (CRD) with six treatments. Four replicates were maintained for each treatment.

***CO_2 efflux*:** For measurements of soil respiration, total CO_2 after 1, 3, 5, 10, 15, 20, 30, 45 and 60 days of incubation was trapped in 1*M* NaOH solution and was measured by titration against 0.01*M* HCl with addition of 2*M* $BaCl_2$ solution and phenolphthalein as indicator (Kuzyakov and Cheng, 2001).

***Soil microbial biomass carbon*:** For determination of MBC, the chloroform fumigation-extraction method was used (Vance *et al.*, 1987). Briefly, 5 g fresh soil was fumigated with chloroform. After 24 h, the fumigated soil samples were extracted with 20 mL of 0.05 *M* K_2SO_4 solution. While 5 g fresh non-fumigated soil was also extracted with 20 mL of 0.05 *M* K_2SO_4. The total C concentration of the obtained extract was analyzed by titrating with 0.033 *M* ferrous ammonium sulfate. In addition, C-CO_2 emissions to soil microbial biomass ratio known as metabolic quotient or qCO_2 was also calculated in normal and saline soils at all moisture levels. The qCO_2 was calculated by using following (Eq. 1).

Metabolic quotient (qCO_2) = CO_2 efflux / Soil MBC (Equation 1)

***Enzyme assays*:** The soil extracellular enzyme activities were measured using fluorogenically labeled substrates, MUF-ß-D-glucopyranoside (EC 3.2.1.21) for ß-glucosidase, MUF-N-acetyl-ß-D-glucosaminide dehydrate (EC 3.2.1.52) for Chitinase, MUF-phosphate monoester (EC 3.1.3.2) for Acid phosphatase and L-Leucine-7-amino-4-methylcoumarin (EC 3.4.11.X) for L-Leucine aminopeptidase (Sanaullah *et al.*, 2011). Briefly, soil suspension was prepared by adding 0.5 g fresh soil in 50 mL of sterilized water and shaking it on a mechanical shaker for 30 minutes. From soil suspension, 50 µL was pipetted in the 96-well microplate. Buffer solutions (50 µL, MES or Trizma) were added in each well containing soil suspension to maintain the pH (6-6.5) and finally, 100 µL of specific substrates (200 µ*M*) were added to make a final volume of 200 µL. The fluorescence was measured after 2 hours at an excitation wavelength of 360 nm and an emission wavelength of 460 nm by using microplate reader

(SYNERGY-HTX, BioTek, USA). Enzyme activities were expressed as MUF or AMC release in nmol per g dry soil per hour (nmol g^{-1} h^{-1}).

***Statistical analysis*:** For comparing soil type and moisture levels, two-way analysis of variance (ANOVA) and least significant difference (LSD) test were performed by using Statistix 8.1. For the quantification of the kinetics of cumulative C-CO_2 effluxes (mg g^{-1} dry soil), SIGMA PLOT 12.5 (Jandel Scientific) was used for data fitting by using single exponential rise to maximum curves (Eq.2.)

$$f = a*(1-\exp^{(-b*t)}) \qquad \text{(Equation 2)}$$

where "f "is cumulative C-CO_2 emissions, "a" is the size of active carbon pool, "b" is first-order kinetic constant for the active C pool and "t" is time in days.

RESULTS

***Soil respiration*:** With increasing moisture stress, cumulative C-CO_2 emissions were significantly increased (Fig. 1). It resulted in increased active C pool (1153±96 mg C kg^{-1} soil) under severe drought stress compared with optimum moisture conditions (Table 1). Similarly, CO_2 emissions were higher under salinity conditions at optimum moisture. While, cumulative C-CO_2 emissions significantly decreased when salinity and drought stress were applied together (Fig. 1). Combined stresses of drought and salinity significantly increased ($p < 0.05$) decay rate constant.

***Soil microbial biomass carbon*:** Soil microbial biomass carbon was significantly increased under drought stress (601.55±79 mg C kg^{-1} soil) as compared with optimum moisture conditions (259.83±37 mg C kg^{-1} soil) (Fig. 2). Similarly, soil MBC was significantly higher under salinity stress. While there was no significant combined effect of drought and salinity (Fig. 2). Same trend was found for metabolic quotients for drought and salinity (Table 1). In contrast, dissolved organic carbon (DOC) significantly decreased under drought and salinity stresses when applied separately but there was no combined effect of drought and salinity on DOC (Fig. 3).

***Extracellular enzymes activities*:** Under drought stress, there was no significant change in C and P degrading extracellular enzyme activities i.e. β-glucosidase, chitinase and acid phosphatase (Fig. 4).

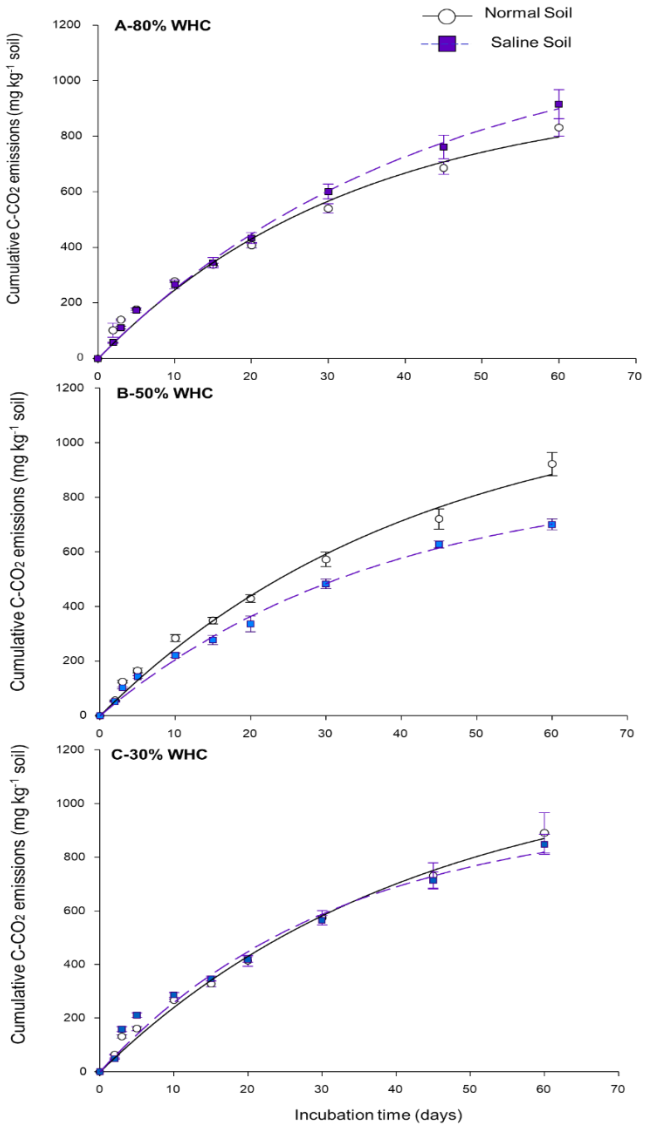

Figure 1. Cumulative C-CO_2 emissions at (A) optimum conditions-80% WHC, (B) moderate drought stress-50% WHC, and at (C) severe drought stress-30% WHC for both normal and saline soils. Data represented as mean ± SE (n=4).

Table 1. Metabolic quotient (qCO_2) calculated by equation 1, active C pool calculated by equation 2 and decay rate constant under drought and salinity stresses. Data represented as mean ± standard error (n=4). Letters with the values indicate significant differences between treatments according to LSD test ($p < 0.05$). LSD values for qCO_2=0.11, active carbon pool=369 and decay constant=0.0036.

Treatments	Metabolic quotient (mg C-CO_2 kg^{-1} soil/mg MBC kg^{-1} soil)		Carbon pool (mg C-CO_2 kg^{-1} soil)		Decay rate constant	
	Normal Soil	Saline Soil	Normal Soil	Saline Soil	Normal Soil	Saline Soil
80 % WHC	0.332±0.015d	1.015±0.054b	956 ± 87b	1184 ± 73a	0.0300 ± 0.005ab	0.0238 ± 0.002b
50 % WHC	0.526±0.041c	1.241±0.127a	1166 ± 11a	878 ± 59b	0.0237 ± 0.0038b	0.0268 ± 0.003ab
30 % WHC	0.610±0.099c	0.981±0.126b	1153 ± 96a	968 ± 91b	0.0234 ± 0.0032b	0.0310 ± 0.006a

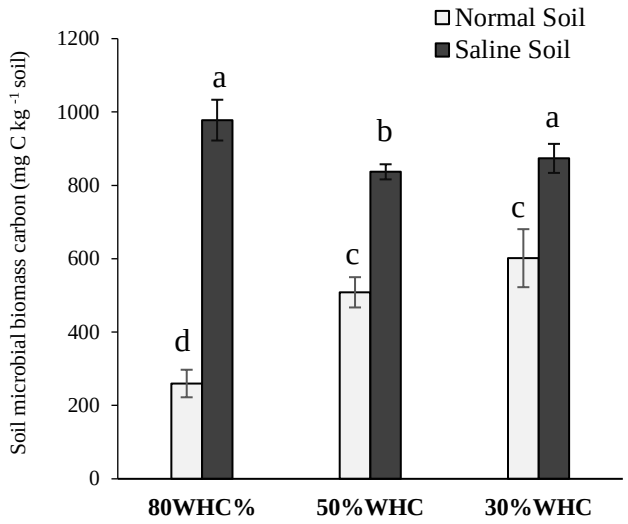

Figure 2. Microbial biomass C at optimum conditions (80%WHC), moderate drought stress (50% WHC), and severe drought stress (30%WHC) for both normal and saline soils. Data represented as mean ± SE (n=4). Letters above the bars indicate significant differences between treatments according to LSD test ($p < 0.05$).

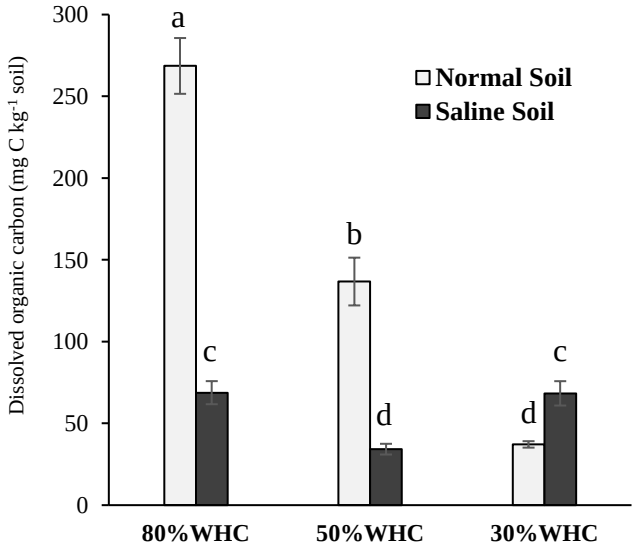

Figure 3. Dissolved organic C at optimum conditions (80%WHC), moderate drought stress (50% WHC), and severe drought stress (30%WHC) for both normal and saline soils. Data represented as mean ± SE (n=4). Letters above the bars indicate significant differences between treatments according to LSD test ($p < 0.05$).

While drought stress resulted in significant increase in leucine aminopeptidase activity (38%) which is peptide degrading enzyme (Fig. 4D). Under salinity stress, there was significant increase in all enzymes except chitinase where there was no signficant change. Combined drought and salinity stresses increased the chitianase enzyme activity (Fig. 4B). In contrast, there was significant decrease in β-glucosidase activity under combined stresses of drought and salinity (Fig. 4A).

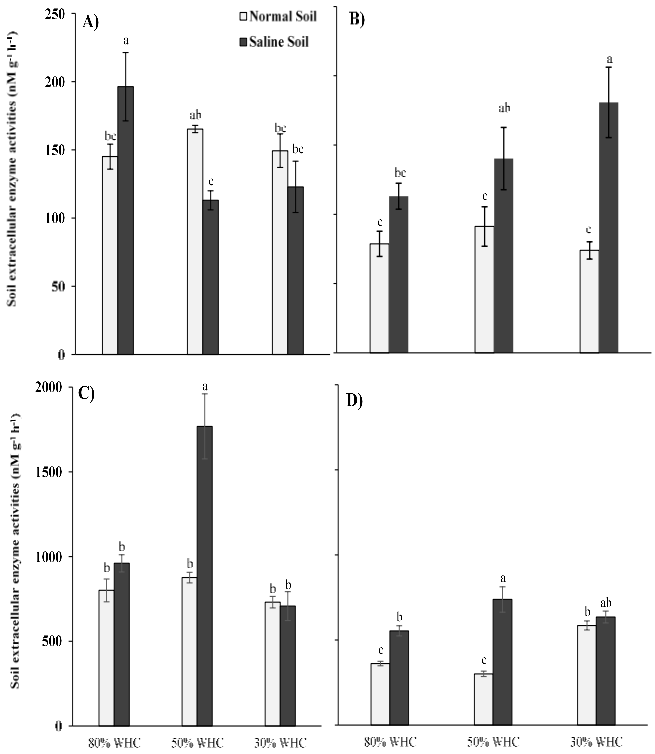

Figure 4. Extracellular enzyme activities (A) β-Gucosidase, (B) chitinase, (C) acid Phosphatase and (D) leucine amino peptidase at optimum conditions (80%WHC), moderate drought stress (50% WHC), and severe drought stress (30%WHC), for both normal and saline soils. Data represented as mean ± SE (n=4). Letters above the bars indicate significant differences between treatments according to LSD test ($p < 0.05$).

***Principle component analysis*:** Principle component analysis of soil health paprameters under drought and salinity stresses explained 83% variablity of the data with first two factors PC1-55% and PC2-28% (Fig. 5). Normal and saline soils were separated in two groups showing different behaviour of microbial activities. Microbial biomass and enzyme activies were positively correlated with principle component and controlling factors for saline soil (Fig. 5). While soil respiration and β-glucosidase activities were correlated with negative axis for normal soils.

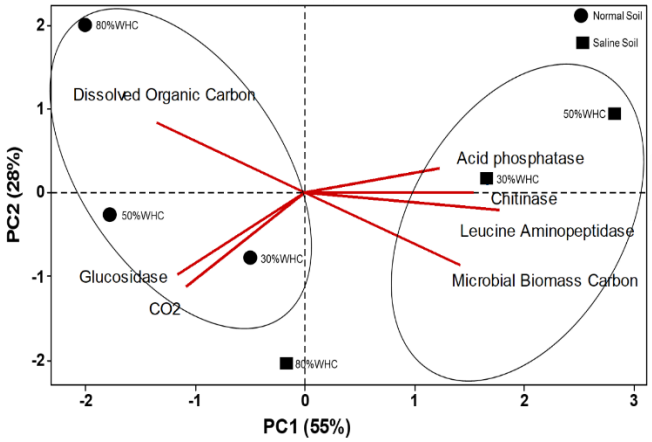

Figure 5. Principle component analysis (PCA) with means of all parameters determined under drought and salinity stresses

DISCUSSION

***Drought effects on soil respiration and microbial activities*:** Our results indicated that under drought stress, increased cumulative C-CO_2 emissions were highly correlated with increased MBC. Similarly, increased metabolic quotient under drought stress also indicated active microbial functioning because microbial communities especially fungi have more resistance to soil drying. It has been reported in literature that MBC often remains stable or even increased under drought conditions (Schaeffer *et al.*, 2017). In addition, there is possibility that under drought stress, soil bacteria can synthesize exopolysaccharides for increasing their biomass. Microorganisms which are tolerant to stress, they accumulate osmolytes, as osmolytes in bacteria are amino acids and polyols can be accumulated in fungi. Microbial cell membrane and cell walls are in close contact with soil. When soils dry and the water potential drops, microbes have to avoid this by making impermeable cell walls to inevitably balance with the water potential around them in the soil (Schimel, 2018). Interestingly, soil respiration was increased under drought stress but there was no significant change in extracellular enzyme activities related to C cycling (β-glucosidase and chitinase) because it is often anticipated that activities of microbes decline due to physiological responses of microbes to moisture stress.

This increased soil respiration may also be correlated with utilization of DOC by microorganism as DOC was signficantly decreased under drought stress. The balance of DOC production and consumption in soil states about its concentration in soil. As DOC contents are the easily decomposable substrates, this enable us to assume that in our study higher metabolism was due to consumption of DOC contents (Yang *et al.*, 2018) because microbes would require more energy for metabolism, as a result more DOC was utilised. Water molecules are stabilized due to these dissolved materials which regulate osmotic potential and help microorganisms to tolerate drought stress. Under drought stress, increased soil respiration may be correlated with depolymerization by extracellular enzymes which enhance the availablity of plant detritus for microbial use (Henry, 2013). Another possibility for regulation of microbial activity under drought stress must be reduction of solute potential by accumulation of osmolytes as solutes (Wood, 2015). Compatible solutes which avoid the disrupting of cell metabolism include glutamate, glycine betaine, proline and trehalose. Bacteria accumulate nitrogenous osmolytes which include glycine betaine and proline while fungi accumulate carbohydrates which include mannitol, erythritol and glycerol (Witteveen and Visser, 1995). There was no change in extracellular enzyme activities or even enhanced L. aminopeptidase activity under drought stress. This is justified as extracellular enzyme are in close contact with their substrates and require thinner water films to function; hence, enzyme activity might be continued with soil drying resulting decline in cellular metabolism (Geisseler *et al.*, 2011).

Another strategy for efficient microbial activity under drought conditions is through production of extracellular polymeric materials. These substances are polysaccharides and protein materials which help in microbial survival under drought stress. Microbial physiological stress and their sensitivity under soil drying can be balanced under increased substrate supply (Schimel, 2018). Some microbial predators depend on water filled pores to reach their microbial prey (Stefan *et al.*, 2014) but as the soil become dry under drought stress, their aptitude to catch the microbial prey fails and microbial death declines. Thus microbes survive under dry conditions and continue their activities.

***Salinity stress impact on soil respiration and microbial activities*:** Salinity showed similar impact like drought stress on soil respiration and microbial activities as cumulative C-CO_2 emissions, MBC and enzyme activities increased under saline conditions. Under saline conditions, soil microbes accumulate exopolysaccharide to resist salinity. Similarly, SOM stabilization and soil aggregate formation are facilitated by enzymatic activities that activate defense mechanisms (Szoboszlay *et al.*, 2019). Increase in CO_2 emission due to salinity stress is because the soil microbial biomass can acclimate to soil environmental conditions (Luo *et al.*, 2019). Increase in soil respiration under salinity stress might be due to adjustment of soil microbes to low osmotic potential as microbes can accumulate osmolytes and were able to avoid water loss from their cells. This is logical because CO_2 emissions depends upon activities of β-glucosidase enzyme involved in C cycle (Mancinelli *et al.*, 2013) and β-glucosidase enzyme activity was significantly increased under salinity stress.

Soil organic matter decomposition is a factor which influences the activity and biomass of microbes in soil.

Metabolic quotient increased with increasing SOM decomposition under salinity stress. Under salinity stress soil bacterial community might be affected and activities of C degrading extracellular enzymes can be enhanced by decreasing SOM (Morrissey *et al.*, 2014). This deceptive difference in drifts with soil MBC may be due to prompted shift of microbial population which dominated with active microorganisms to those which may be less active. Like drought stress, DOC contents were significantly decreased under salinity stress; this relies on soil CO_2 emissions and MBC (Jinbo *et al.*, 2007) because under salinity stress, CO_2 emissions and MBC were increased which exhausted the available DOC contents.

Increase in soil extracellular enzyme activities may be ascribed due to higher MBC. Under higher salt concentration, the salt-tolerant microbes show increased soil extracellular enzyme activities by synthesizing osmolytes (Slama *et al.*, 2015), modifying bacterial morphology like changes in cell volume, cell elongation and cell shrinkage, and changing synthesis pattern for polysaccharides, proteins, fatty acids and lipids.

***Combine effects of drought and salinity on soil respiration and microbial activities*:**

In contrast to individual stresses, combined stresses of drought and salinity resulted in significant decrease in soil respiration which could be correlated with decreased β-glucosidase activity. Microbial activities decrease under interactive effect of drying and salinity stresses due to low osmotic potential (Yemadje *et al.*, 2017). This correlation was confirmed in principle component analysis (Fig 5). Severe lack of moisture might be the reason for this decrease in microbial activities because during such dry conditions in presence of salinity, transport of nutrients can be limited, as a result starvation may occur (Baumann and Marschner, 2013). It has been reported that co-occurrence of salinity and drought aggravated osmotic stress which caused low microbial activities. Similarly, many microorganisms go dormant in highly unfavorable conditions (Jones and Lennon, 2010) due to which microbial activities are decreased and this effect was observed under combine drought and salinity stresses.

Under combined stresses of drought and salinity, DOC contents showed no significant difference compared with optimum conditions which indicates that microorganisms were unable to utilize this easily available energy source under dual abiotic stresses. This was consistent with MBC data where there was no combined effect of drought and salinity. Under combined abiotic stresses, higher chitinase activities confirmed the availability of chitin from microbial dead biomass degradation due to severe abiotic stresses (Kreyling *et al.*, 2008). The present study addresses that the soil biological activities were suppressed under combined effects of drought and salinity. There was no adaptation mechanism of soil microbes for their activities under soil drying in saline soils. However, under individual stresses of drought and salinity, soil microbes showed adaptive mechanisms and soil biological activities were increased.

***Conclusion*:** It was concluded from our study that soil respiration was significantly increased when drought and salinity stresses were applied separately indicating adaptation of soil microbes to individual stresses of drought and salinity. This increased cumulative C-CO_2 was highly correlated with increased MBC and decreased DOC as easily degradable C source was used by soil microbes for their activities. Regarding extracellular enzyme activities, under salinity stress, there was significant increase in all enzymes except chitinase where there was no signficant change. While combined abiotic stresses of drought and salinity has detrimental effects on cumulative C-CO_2 emissions and microbial activities indicating limited microbial activities under combined stresses of drought and salinity. Chitianase activity increased under combined abioitic stresses, confirming the availability of chitin from microbial dead biomass degradation. Thus climate change stress factors (drought and salinity) combined limit the normal soil biological processes cause to limit the nutrient cycling in soil ecosystem and as a result loss of soil health.

***Declaration of competing interest*:** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

***Authors contributions*:** Abdul Qadeer and Muhammad Sana Ullah have conceived and planned the study. Abdul Qadeer wrote the paper with input from all the authors. Abdul Wakeel and Sardar Alam Cheema helped to improve the draft.

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